WATER RESOURCES RESEARCH, VOL. 31, NO. 7, PAGES 1627–1636, JULY 1995

Bacterial sedimentation through a porous medium

Jiamin Wan, Tetsu K. Tokunaga, and Chin-Fu Tsang
Earth Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley

Abstract. Numerous previous studies of bacterial transport in groundwaters and to deep aquifers and sediments have either neglected, or regarded as insignificant, the potential contribution of bacterial sedimentation. This study examines the potential significance of sedimentation as a mechanism for bacterial transport. A simple model is developed to predict the behavior of particles (bacterial or inorganic colloids) sedimenting through granular porous media under hydrostatic conditions. The model indicates that tortuosity-limited sedimentation velocities through porous media consisting of large, well-rounded grains can proceed at velocities close to (≈90% that of) free sedimentation in water columns when particle-grain interactions involve only tortuosity. The assumption of neutral buoyancy of bacteria was demonstrated to be invalid through buoyant density measurements on 25 subsurface bacterial strains (using Percoll density gradient centrifugation). An average buoyant density of 1.088 Mg m^{-3} was obtained (range from 1.040 to 1.121 Mg m^{-3}). The two nonmotile bacterial strains selected for sedimentation experiments were *Arthrobacter globiformis* B672 (isolated from the Middendorf aquifer, 259-m depth), and OYS3, a streptomycin-resistant strain isolated from shallow groundwaters at Oyster, Virginia. All experiments were carried out under nongrowth conditions. Stokes' law sedimentation velocities for the two bacterial strains calculated from measurements of buoyant densities and characteristic sizes were 5.8 and 40 mm d^{-1}, respectively. Direct measurements of free sedimentation of *Arthrobacter* B672 and OYS3 through water columns (21°C) yielded median velocities of 7.1 and 42 mm d^{-1}, respectively, in good agreement with Stokes' law calculations. The *Arthrobacter* B672 and OYS3 strains sedimented through saturated sand columns (quartz sand, 300–420 μm diameter) under hydrostatic conditions at median velocities of 7 and 17 mm d^{-1}. Thus the sedimentation model is consistent with sand column observations on *Arthrobacter* B672 and too simplistic in the case of OYS3. Bacterial breakthrough by sedimentation exhibited trends consistent with first-order attenuation with distance. Bacterial deposition coefficients for this first-order model were in the range of 0.008–0.012 mm^{-1}. Surface physical-chemical interactions, grain and pore size distributions, and grain surface microtopography can be very important in controlling the effectiveness of bacterial sedimentation as a transport mechanism. This research suggested that if timescales are sufficiently long, spanning many generations, sedimentation can become a significant mechanism for bacterial transport.

Introduction

Concerns about propagation of pathogenic microbes in groundwaters [e.g., *Gerba et al.*, 1975] and the more recent use of microorganisms for in situ contaminant degradation in aquifers [e.g., *National Research Council*, 1993] and for petroleum recovery [e.g., *Jang et al.*, 1983] have motivated research directed toward better understanding of how microorganisms (either indigenous or injected from above ground) move through the porous medium. An appreciation of bacterial transport mechanisms is also important in studies of deep subsurface microbial populations [*Fredrickson et al.*, 1988; *Balkwill et al.*, 1989; *Pedersen and Ekendahl*, 1990; *Haldeman and Amy*, 1993; *Kieft et al.*, 1993; *Pedersen*, 1993]. A number of theoretical and experimental studies have been performed to understand mechanisms controlling bacterial transport in subsurface environments [e.g., *Corapcioglu and Haridas*, 1984; *Yates and Yates*, 1988; *Harvey et al.*, 1989; *Harvey and Garabedian*, 1991; *Harvey*, 1991; *Fontes et al.*, 1991; *Hornberger et al.*, 1992; *Martin et al.*, 1992]. Research on subsurface bacterial transport has borrowed extensively from earlier developments in filtration theory [e.g., *Yao et al.*, 1971; *Rajagopalan and Tien*, 1976; *Tien and Payatakes*, 1979; *Rajagopalan and Chu*, 1982]. Results from most previous analyses imply that particle transport ceases when advection of pore waters is absent. Mechanisms common to both bacterial transport and colloid filtration processes include advection, dispersion, and deposition. The latter process can be subdivided into the more specific mechanisms of straining, Brownian diffusion, interception, and sedimentation, with subsequent sorption-desorption. Important influences of groundwater chemistry and surface physical chemistry of bacteria and porous media on sorption have also been emphasized in previous studies [*Stenstrom*, 1989; *Gannon et al.*, 1991; *Lindqvist and Bengtsson*, 1991; *Scholl and Harvey*, 1992; *Huysman and Verstraete*, 1993; *Wan et al.*, 1994]. In bacterial transport models, additional mechanisms of motility, chemotaxis and tumbling, as well as growth and death can also be included [*Jenneman et al.*, 1985; *Corapcioglu and Haridas*, 1984, 1985; *Sharma et al.*, 1993].

Paper number 95WR01255.
0043-1397/95/95WR-01255$05.00

Sedimentation can potentially contribute to either local deposition or downward movement of suspended particles. In a study of bacterial sedimentation in lakes, *Jassby* [1975] measured free "sinking" rates of a subalpine lake planktonic bacterial strain (0.66 ± 0.07 μm diameter, buoyant density 1.020 ± 0.001 Mg m^{-3}) under hydrostatic laboratory conditions. The measured values fell between 0.5 and 3.2 mm d^{-1}. The Stokes' law predicted values were 0.6–2.2 mm d^{-1}. *Pedros-Alio et al.* [1989] measured in situ "sinking" rates of phototrophic bacteria in Lake Ciso over a 2-year period. They reported that free-living microorganisms "sink" at speeds predicted by Stokes' law under nonturbulent conditions. Although sedimentation has been studied in surface waters, and studied prominently in filtration as a mechanism for deposition, it has received much less attention in studies of bacterial transport in porous media. Specific gravities of bacteria ranging from 1.04 to 1.13 have been used for calculating the contribution of sedimentation to the single-collector efficiency parameter by *Martin et al.* [1992]. The assumption of neutral buoyancy, i.e., that bacterial densities are similar enough to densities of groundwaters so that sedimentation is negligible, has been mentioned in some previous work. *Corapcioglu and Haridas* [1985] and *Yates and Yates* [1988] note that viruses and some bacteria are neutrally buoyant. Under conditions of high flow rates and short timescales, neutral buoyancy of bacteria is a reasonable approximation [*Hornberger et al.*, 1992]. Under more general field conditions, uncertainties associated with this assumption have previously been identified by *Harvey and Garabedian* [1991]. The phenomenon of bacteria sedimentation has been shown to contribute to errors in laboratory assays of motility and chemotaxis by *Shonnard et al.* [1992]. The possibility that sedimentation may be a ubiquitous yet unrecognized mechanism for bacterial transport appears worthy of investigating. This mechanism may be of equal or greater importance than diffusion-dispersion in studies of long-term downward bacterial transport since displacements due to sedimentation are linear with respect to time rather than dependent as the square root of time. Thus sedimentation may contribute to explanations for the presence of bacterial populations found in deep subsurface environments.

The present study was initiated to reexamine the potential significance of bacterial sedimentation in porous media. First, an analysis is performed to estimate the influence of a simple granular porous medium on bacterial sedimentation. The experiments described in the sections which follow begin with determinations of densities and sizes of bacteria and then proceed to studies of bacterial sedimentation in static water columns (termed free sedimentation in this study) and bacterial sedimentation in hydrostatic, water-saturated sand columns. In all of these experiments, only resting stage, nonmotile bacteria were studied under nongrowth conditions.

Theory

For the purpose of isolating bacterial sedimentation as a transport mechanism, the following analysis and experiments all involve hydrostatic conditions. We further restrict this study to cases where the mineral grains which compose the porous media are well rounded and much larger than the sedimenting particles (bacteria or inorganic solids), typically by a factor of at least 100×. For sedimentation of colloid-size particles through granular porous media, the simplest case to consider involves no frictional interactions between particles and mineral grains. The motion of particles is idealized as alternating between free sedimentation through pores and sliding down upper hemispheres of smooth mineral grains. This simplest starting point permits estimation of the maximum possible sedimentation velocity through porous media. Mechanisms causing immobilization of particles (straining, interception, and various sorption mechanisms) are later idealized as operating uniformly and irreversibly throughout the porous medium. Reversibility in these mechanisms modifies analyses of sedimentation velocities, but the required additional kinetic information or assumptions are beyond the present scope of investigation. In the following analyses we are concerned primarily with average velocities of particles in response to gravity, rather than thermal motion.

Since sedimenting particles are forced to traverse tortuous pathways, it is necessary to determine the timescales and space scales over which accelerations and decelerations occur. If these scales are short enough, then local steady state velocities which are uniquely determined by instantaneous trajectories may be assumed. We approach this problem by beginning with the equation of motion for a sphere moving at an arbitrary (but low Reynolds number) instantaneous velocity. The motion of a spherical particle of diameter D and density ρ_s sedimenting at low Reynolds numbers is given by Newton's second law, where the particle mass times its acceleration is equated to the sum of instantaneous forces. The viscous, gravitational, and buoyant forces are thus related to particle acceleration by

$$\left(\frac{\pi}{6}\rho_s D^3\right)\frac{dv}{dt} = -3\pi\eta D v - \frac{\pi}{6}(\rho_s - \rho_f) g D^3, \qquad (1)$$

where v is the instantaneous velocity, η is the dynamic fluid viscosity, ρ_f is the fluid mass density, g is the acceleration of gravity, and t is time. The solution to (1) for an initial velocity v^* is

$$v(t) = -\frac{1}{18\eta}(\rho_s - \rho_f) g D^2 + \left(v^* + \frac{1}{18\eta}(\rho_s - \rho_f) g D^2\right) \exp\left(\frac{-t}{\tau}\right), \qquad (2)$$

where the damping time constant is

$$\tau = \rho_s D^2/18\eta. \qquad (3)$$

This parameter provides an indication of the length of time necessary for dissipation of nonsteady state velocities. For colloid-size particles in ambient temperature water, τ is very small. For 1-μm-diameter particles with ρ_s of 1.00 and 2.65 Mg m^{-3}, τ equals 5.6×10^{-8} and 1.5×10^{-7} s. In an analysis of a slightly different problem of bacterial motion, *Berg* [1993] demonstrated that velocity perturbations are damped over times less than 1 μs and distances of less than 0.1 nm. Our derivation is consistent with this conclusion. For $t > 5\tau$, the exponential term becomes insignificant, leaving the well-known steady state result of

$$v_0 = (1/18\eta)(\rho_s - \rho_f) g D^2. \qquad (4a)$$

In view of the shortness of τ, we will approximate all local sedimentation velocities by steady state values calculated in the following manner. For a particle sedimenting through a porous medium without attractive interactions with grain surfaces, (4a) represents the maximum possible value of its instantaneous velocity. The generally tortuous path which the sedi-

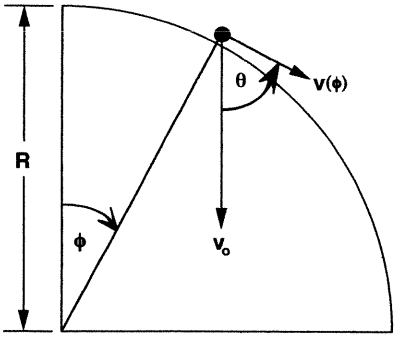

Figure 1. Spherical particle sliding down upper hemisphere of a grain surface.

menting particle must take through the porous medium causes its instantaneous velocity to deviate from the direction of gravitational acceleration, thereby resulting in

$$v(\theta) = \varepsilon v_0 \cos \theta, \tag{4b}$$

where θ represents the angle between the instantaneous velocity and the direction of gravitational acceleration and ε is an empirical correction factor arising from influences of the grain surface ($0 \leq \varepsilon \leq 1$). In the simplest case this factor arises from the stationary fluid boundary associated with the grain surface. In more general models the wall correction factor ε could also include the influence of particle rotation, the presence of other walls (grains), and θ-dependent particle-grain friction [*King et al.*, 1992]. With $\theta \neq 0$, (4b) approximates instantaneous sedimentation velocities of a particle as it slides downward over "upper hemispheres" of mineral grains. The $\theta = 0$ and $\varepsilon = 1$ condition represents local free sedimentation through portions of a pore, applicable to the vertical interval bounded by the "equatorial edge" of its previously encountered grain and the surface grain segment directly below it. From this qualitative consideration it is apparent that a tortuosity factor for particle sedimentation through a porous medium is different from the tortuosity concept associated with either diffusion or advective flow. Under hydrostatic conditions, non-Brownian sedimenting particles traverse porous media via a small fraction of the pore space.

The problem of deducing the average sedimentation velocity of a particle through a porous medium remains quite dependent on details of distributions of grain shape, grain size, grain orientation, porosity, and pore geometry. It is instructive to consider the simple reference model of smooth spherical grains (of radius R) onto which colloidal particles fall randomly. We first consider the influence of particles sliding downward on individual grain surfaces and then bring in influences of short free sedimentation segments through individual pores. It will be convenient to utilize the spherical coordinate system shown in Figure 1. The angle ϕ is related to θ by $\phi = (\pi/2) - \theta$. We consider a particle sliding downward over the hemisphere, at an instantaneous angular position ϕ ($0 \leq \phi \leq \pi/2$). Equation (4b) may be reexpressed in terms of ϕ as

$$v(\phi) = \varepsilon v_0 \sin \phi \tag{4c}$$

so that

$$\frac{d\phi}{dt} = \frac{\varepsilon v_0}{R} \sin \phi, \tag{5a}$$

or

$$\frac{d\phi}{dt} = \frac{\varepsilon}{T_0} \sin \phi \tag{5b}$$

where T_0 is the free sedimentation time needed to traverse the vertical distance R. Equation (5b) may be integrated from an initial particle position of ϕ_i to $\pi/2$, where the particle rolls off the grain equator. In this manner we obtain the time $T(\phi_i)$ needed for the particle to slide from position of ϕ_i to $\pi/2$,

$$T(\phi_i) = \frac{T_0}{\varepsilon} \int_{\phi_i}^{\pi/2} \frac{d\phi}{\sin \phi} \tag{6a}$$

or

$$T(\phi_i) = \frac{T_0}{\varepsilon} \ln \left(\cot \frac{\phi_i}{2} \right). \tag{6b}$$

Normalization of this result to the free sedimentation time to fall the equivalent vertical distance of $R \cos(\phi_i)$ gives

$$T^*(\phi_i) = \frac{1}{\varepsilon \cos(\phi_i)} \ln \left(\cot \frac{\phi_i}{2} \right) \tag{7}$$

Determining the average value of T^* for all particles sliding down the hemisphere requires an assumption concerning the probability distribution of ϕ_i from the sedimenting particles falling onto the model grain. Approximating the distribution of particles falling onto the grains as uniform amounts to a projected area-weighted probability of particles colliding with grains within $d\phi$ of ϕ_i given by

$$dp = 2 \sin \phi_i \cos \phi_i \, d\phi. \tag{8}$$

The average value of T^* is then obtained as

$$\overline{T^*} = \frac{2}{\varepsilon} \int_0^{\pi/2} (\sin \phi) \ln \left[\cot \left(\frac{\phi}{2} \right) \right] d\phi \tag{9a}$$

and

$$\overline{T^*} = (2/\varepsilon) \ln 2 \approx 1.386/\varepsilon. \tag{9b}$$

Recall that these calculations have been concerned only with the portions of particle trajectories which involve sliding down grain surfaces and do not include sorption. The average vertical distance traversed by these trajectories is $(2/3)R$. Over this interval the average sliding sedimentation time is $0.386/\varepsilon$ longer than that of the corresponding free sedimentation time. The effective vertical component of the velocity is therefore about 0.722ε that of free sedimentation. However, visual inspection of simple close-packed spheres indicates that the presence of overlying grains may minimize the probability of very small ϕ_i values which contribute the larger values of T^*. We have also ignored free sedimentation trajectories which bypass the upper hemisphere and fall freely through the plane of pores located at $\phi = \pi/2$. These factors will bring average sedimentation velocities closer to free sedimentation rates. Also, since the segments of free sedimentation through individual pores below the equatorial plane ($\pi/2 < \phi < \pi$) are not accounted for in (9b), the above result further underestimates the average, tortuosity-limited sedimentation velocity in this model porous medium. The free sedimentation segments are on average about equal to R. The average sedimentation velocity in the porous medium can be calculated as the harmonic

average of sliding and free sedimentation velocities. This average is approximately

$$\bar{v} \approx \frac{bR + \frac{2}{3}R}{(bR/v_0) + (\frac{2}{3}R/0.721\varepsilon v_0)} \quad (10a)$$

or

$$\bar{v} \approx \left(\frac{b + 0.67}{b + (0.93/\varepsilon)}\right) v_0, \quad (10b)$$

where $b \approx 1$ (representing the ratio of the average free sedimentation segment length to the grain radius). Equation (10b) is fairly insensitive to the value of b (within reasonable lower limits). For $b \geq 1$ and $\varepsilon = 1$, we have $0.86v_0 \leq \bar{v} \leq v_0$. Even for $b = 0.5$ and $\varepsilon = 1$, $\bar{v} \approx 0.82v_0$. Thus the upper limit of average particle sedimentation through saturated porous media composed of well-rounded grains is about 90% that of free sedimentation when grains contribute only to tortuosity and do not impart additional frictional resistance.

Bacteria and other colloidal particles typically experience various depositional processes at grain surfaces, which can prevent their further migration within porous media. For particle sedimentation through a homogeneous porous medium, deposition may be approximated as a first-order process. From this perspective, particle deposition within a porous medium is analogous to the attenuation of photons in an absorbing medium. For an initial total mass M_0 of sedimenting particles entering the porous medium at $z = 0$, the mass that traverses a vertical distance z is given by

$$M(z) = M_0 \exp(-\mu z) \quad (11)$$

where μ is an empirical deposition coefficient (L^{-1}) and z is taken positive downward here to emphasize the analogy to Beer's law radiation attenuation. This type of deposition model has previously been used extensively in filtration models of flowing systems [e.g., *Tien and Payatakes*, 1979]. It is worth noting here that in the hydrostatic limit, filtration models generally predict that no particle transport will occur. However, in this present study we address the possibility that sedimentation is both a mechanism for transport through and deposition within hydrostatic porous media. The analysis presented here further shows that average particle sedimentation velocities through hydrostatic porous media could be quite similar (≈90%) to corresponding free sedimentation rates when only tortuosity influences are important.

Materials and Methods

Bacterial Strains

Twenty-five bacterial strains, all isolates from soils or groundwaters, were obtained from researchers cited in the acknowledgments. These bacterial strains are listed in Table 1 and were all used in determinations of densities. While the majority of these strains have been identified, several isolates from the Oyster, Virginia, site (a field site for the U.S. Department of Energy, Subsurface Sciences Program) are currently being identified (A. Palumbo, Oak Ridge National Laboratory, personal communication, 1994). Two of the bacteria strains from Table 1 were used for experiments on sedimentation. These were *Arthrobacter globiformis* B672, and OYS3 (a streptomycin-resistant strain). Microphotographs of these bacterial strains are shown in Figure 2. *Arthrobacter* B672 was isolated from a depth of 259 m in the Middendorf aquifer [*Balkwill et al.*, 1989]. Strain OYS3 was isolated from shallow groundwaters at Oyster, Virginia [*Palumbo et al.*, 1994].

Table 1. Bacterial Densities

Bacterial Strain	Density, Mg m^{-3}	s.d., Mg m^{-3}
Arthrobacter globiformis B672	1.117	0.007
A. B776	1.068	0.005
A. spp. H12H212	1.098	0.009
A. sp. ZAL001	1.121	0.005
A. sp. S-139	1.064	0.016
A. sp. S-5	1.110	0.003
Pseudomonas cepacia 3N3A	1.109	0.004
P. fluorescens 17579	1.105	0.014
P. fluorescens Pf0-1	1.097	0.008
P. fluorescens Pf1-1	1.095	
P. indigofera 17583	1.104	0.013
P. putida Pp1-1	1.102	0.010
P. putida mt-2	1.073	0.008
P. stutzeri	1.107	0.004
P. sp. OQ strain DSF	1.085	0.005
Bacillus pumulis	1.108	0.006
Agrobacterium tumefaciens NTI	1.092	0.001
OYK	1.058	0.008
OYCu2	1.082	0.007
OYS1	1.068	0.003
OYS2	1.061	
OYS3	1.074	0.009
OYN2	1.040	0.004
OYN3	1.091	0.002
OYBsalt	1.076	0.011

All "OY" bacterial strains were recently isolated from the Department of Energy Subsurface Sciences Program Oyster (Virginia) field site, and have not yet been characterized. Here s.d. denotes standard deviation.

Bacteria were grown in a 10% PTYG broth at 27°C on a shaker. The broth contained the following ingredients per liter of distilled water: 1.0 g of glucose, 1.0 g of yeast extract, 0.5 g of peptone, 0.5 g of tryptone, 0.6 g of $MgSO_4 \cdot 7H_2$), and 0.07 g of $CaCl_2 \cdot 2H_2O$. The cultures were harvested after 48 hours of growth. The cells were harvested by centrifugation and washed in a sterile solution of 2 m*M* NaCl solution (*p*H 6.8) for the later experiments. We emphasize that these bacteria were not experimented with under starving conditions which are likely to be more representative of natural groundwater environments.

Buoyant Density Measurements

The term buoyant density is used here to denote the bacterial mass per unit volume of bacterial cells. To prepare a cell suspension for centrifugation, the washed cells were resuspended in a 0.15 *M* NaCl solution to a concentration of about 1×10^9 cells mL^{-1}. Bacterial buoyant densities were measured within 24 hours after harvesting. Density gradient centrifugation is an established method for the separation and purification of cells, viruses, and subcellular particles [*Pertoft and Laurent*, 1968; *Wolff*, 1975]. Percoll, a commercially available (Sigma Chemical Company) colloidal silica suspension (density, 1.131 g/mL) was used as the gradient medium. Density marker beads (Sigma Chemical Company) were used for indicating specific density values along the density gradients. To generate a preformed, self-generated gradient, the stock Percoll solution was diluted with 0.15 *M* NaCl solution in a 15-mL centrifuge tube to a density chosen in the middle of the range

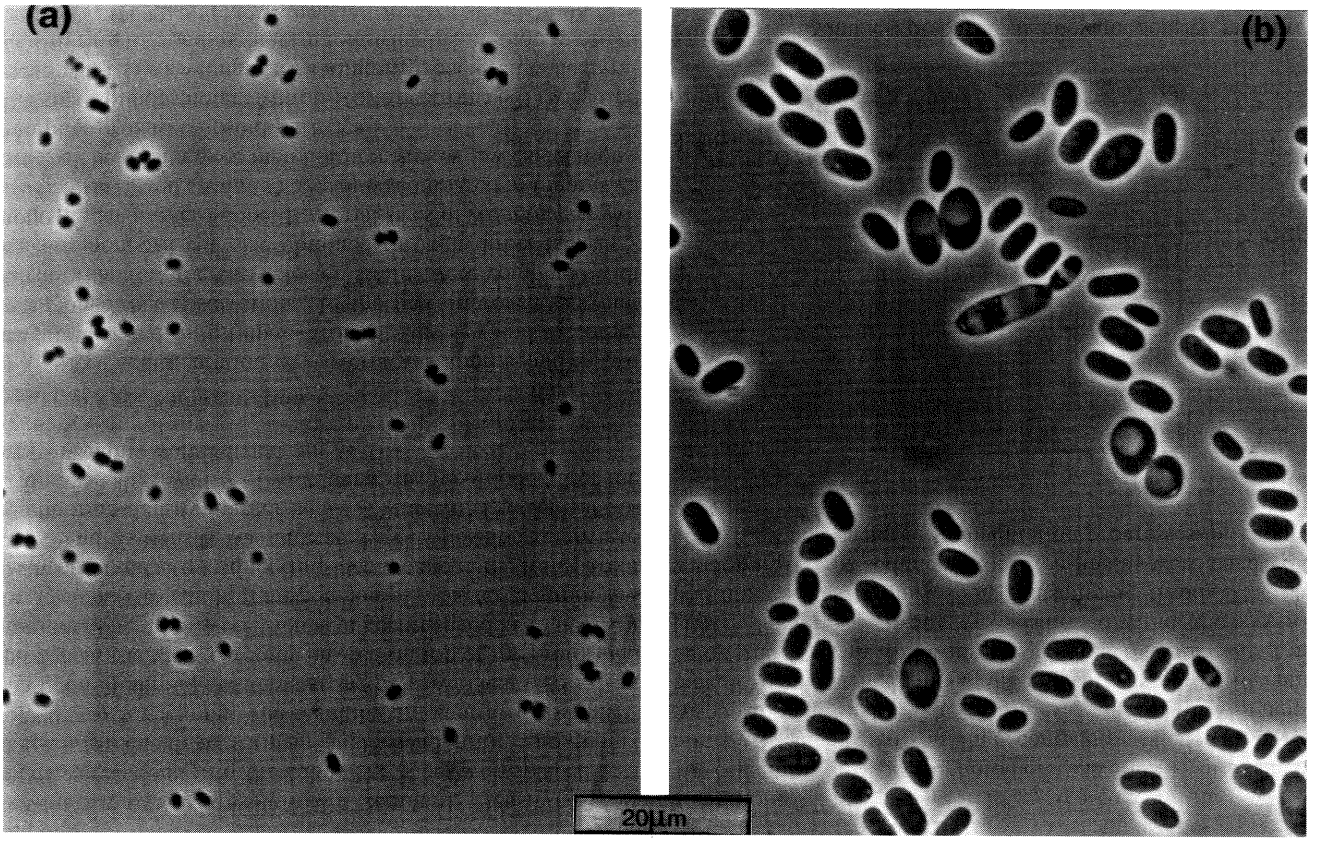

Figure 2. Microphotographs of bacteria strains (a) *Arthrobacter* B672, and (b) OYS3. The scale bar indicates 20 μm.

in which maximum resolution is required. Density marker beads were added into the tube. The tubes were placed in a bench top centrifuge with an angle head rotor, and run at 15,000 $\times$ g for 30 min. The resulting gradient forms symmetrically on either side of the starting density. Then 0.25 mL of cell preparation was carefully layered on top of the preformed gradient. The tubes were then again centrifuged at 15,000 $\times$ g for 15 min. The equilibrium position of a bacterial strain was easily observed as a distinct translucent band in the transparent tubes, and its density was indicated by its position relative to neighboring density marker beads (Figure 3). The central position as well as widths of each band was recorded. All of the measurements were carried out at 21°C.

Bacterial Free Sedimentation

The free sedimentation of bacteria was approached in two ways, one based on estimates from Stokes' law and the other based on direct observations. In the first approach, buoyant density values of the two selected strains were combined with measurements of cell size obtained through direct measurements under a microscope. Bacterial sizes of *Arthrobacter globiformis* B672 and strain OYS3 can be compared in Figures 2a and 2b. These data were then used for Stokes' law calculations, assuming prolate ellipsoid particles.

A spectrophotometer (Milton-Roy Spectronic 601) was used at the 480-nm wavelength for monitoring bacterial concentrations during free sedimentation following the method of *Shonnard et al.* [1992]. Light absorbance versus bacterial numbers was determined to be linear over the experimental range ($\leq 1 \times 10^8$ cells mL^{-1}). Washed bacteria were resuspended in a solution of 2 mM NaCl (pH 6.8) to a concentration of 1×10^8 cells mL^{-1}. A spectrophotometer cuvette (45 mm tall, 10 mm by 10 mm base) was then completely filled with a cell suspension and sealed. The cuvette was masked off with black tape, leaving matched pairs of 0.5-mm slit windows at 10.0 mm below the top rim, following the method of *Shonnard et al.* [1992]. The sealed cuvette was positioned within the spectrophotometer to maximize light transmission through the slit windows. All free sedimentation experiments were conducted at 21°C. When bacterial sedimentation progresses far enough

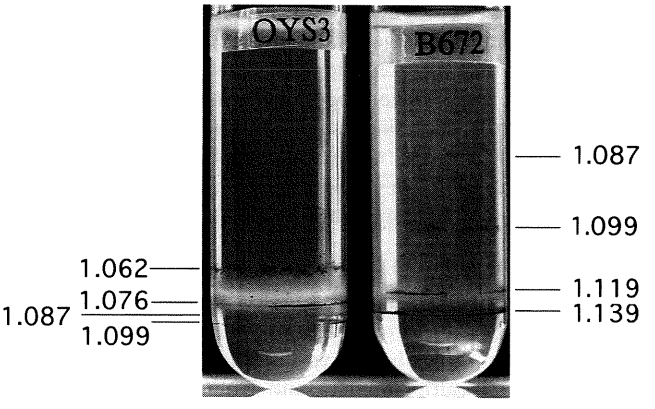

Figure 3. Examples of bacterial buoyant density measurements. The white band in each tube is composed of bacteria at equilibrium positions. The density gradient is indicated by a series of density markers.

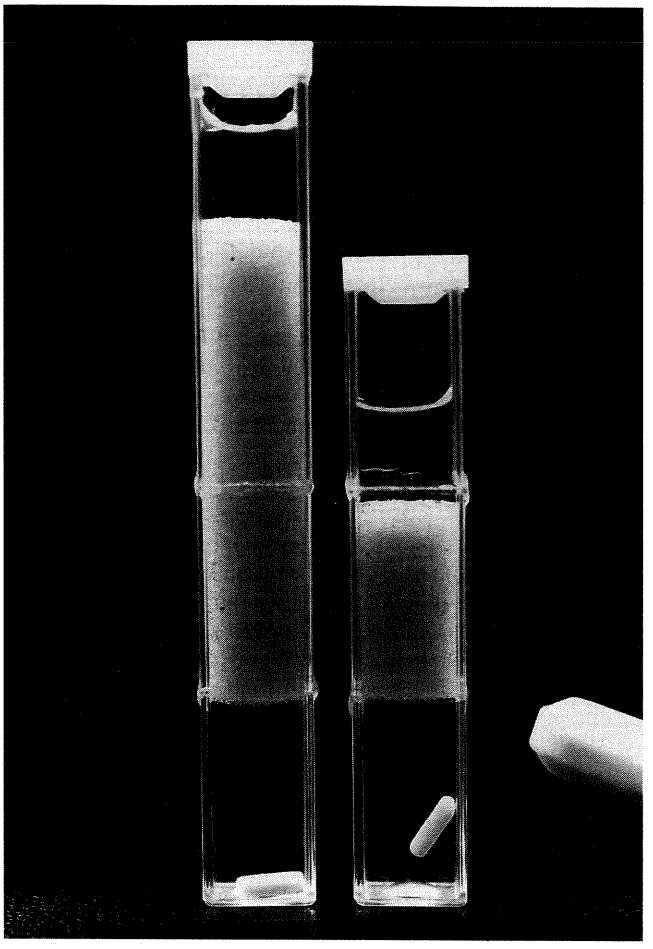

Figure 4. Photograph of 50-mm and 20-mm sand columns for bacterial sedimentation measurements.

so that bacterial concentrations begin to diminish at the 10.0-mm depth, light absorption and scattering within the cuvette also decrease. The time dependence of the spectrophotometer absorbance can then be used to estimate sedimentation velocity distributions. Combining this with measured densities permits calculations of size distributions in a manner typically used in soil particle size analyses [e.g., *Gee and Bauder*, 1986], although this is not a purpose of the present study. An assumption invoked in the present procedure is that of particle size–independent light absorbance. We recognize that this assumption is generally incorrect, most basically because absorbance cross sections of particles are size dependent. However, since size ranges of the individual strains were relatively narrow and since separate absorbance calibrations were not obtained for different sized bacteria within each strain, the aforementioned assumption was used in order to obtain estimates of sedimentation velocity distributions.

Sedimentation Through Saturated Sand Columns

Experiments were conducted under nongrowth and hydrostatic conditions in porous media to isolate the influence of sedimentation rate on the vertical transport of bacteria. Accusand (high-purity quartz sand from Unimin Company, Ottawa, Minnesota) in grain sizes ranging from 300 to 420 μm in diameter was used to pack columns. In order to gain clean grain surfaces and avoid any possible mineral colloids being released from grain surfaces, the sand was washed thoroughly following the procedure by *Wan et al.* [1994], and sterilized before drying. The structure of the columns is shown in the Figure 4 photograph. Polystyrene cuvettes (100 mm^2 cross-sectional area) were used to construct the columns. Polystyrene cuvettes have an optical window to ensure maximum transmittance from 340- to 800-nm wavelengths. A polypropylene screen (mesh opening 105 μm) was inserted into each cuvette at a distance of 22 mm from the bottom. A Teflon-coated magnetic micro stir bar (2 × 7 mm) was kept in the bottom portion of each column, below the polypropylene screen. Each column was soaked in isopropyl alcohol and then rinsed thoroughly with a sterile solution. Each column was then filled with the sterile solution of 2 m*M* NaCl (*p*H 6.8). In each column, precalculated masses of clean, dry sand were poured through the water column to pack above the screen. To minimize sorting of grains during packing, the sand was added in small increments. All columns were packed to a porosity of 0.34 ± 0.01. Columns were packed to two lengths, 20 and 50 mm. The supernatant solution was removed until only a 5-mm layer remained ponded over each sand pack. A 5-mm layer of bacterial suspension (approximately 2 × 10^8 cells mL^{-1}) was then carefully added to the free water surface of each column with minimal disturbance of the pore waters in the sand pack. After gently mixing the two supernatant liquids in each column, a 10-mm layer of bacterial suspension with concentration about 10^8 cells mL^{-1} was obtained, establishing the start of sedimentation. Tops of columns were sealed to avoid evaporation. Columns were placed in a cuvette holder and set in a room with the temperature of 21° ± 1°C. A spectrophotometer (Milton-Roy model 601) was used to periodically measure the optical density (at the 480-nm wavelength) of the solution under each sand column. Before each measurement the bottom reservoir was thoroughly mixed by circulating the micro stir bar with a larger external magnet. The readings of light absorbance at time zero were taken as the background. All of the initial readings were very similar to absorbance of bacteria-free 2 m*M* NaCl solutions. Readings were taken at about 4–10 hour intervals. Three replicates were tested for each combination of bacterial strain and column length. To monitor background changes over time, two blank columns were run, without addition of bacteria. These blank columns were used to detect possible microbial contamination and/or colloid release from sand surfaces. The results showed constant absorbance (equal to the blank solution absorbance) over the entire experimental time. Optical densities of control bacterial suspensions were also measured throughout the experimental period in order to detect any potential changes in absorbance (resulting from decay, growth, aggregation, or starvation). The experiments were carried out for 7 days for strain OYS3, and 10 days for the *Arthrobacter* B672. At the end of the experiments the solutions above, below, and inside the sand packings were sampled. Any bacteria recovered in the postexperiment sampling were checked under the microscope for gross morphological changes.

Results and Discussion

Buoyant Densities

The measured buoyant densities of 25 subsurface bacterial strains are shown in Table 1. Each reported density value is an average of three to seven measurements. Each measurement was from a separate culture, grown at a different time. The replicates from a particular batch always had the identical

range of densities, but from batch to batch there were slight differences. In the density measurements all bacteria were from a late logarithmic stage of growth (48 hours). The uncertainties in the data listed in Table 1 include two factors. One is the width of a bacterial band in a preformed density gradient. The bandwidth varied from strain to strain. For instance, *Arthrobacter* strain B672 had narrow bands ranging between 1.115 and 1.121 Mg m^{-3}. However, strain OYS3 had a wider range of 1.069–1.080 Mg m^{-3}. Some strains had the tendency to form aggregates in the saline solution, such as OYBsalt, and Arthrobacter S139. The second source of variation was the difference from batch to batch. Replicates from the same batch always yielded identical buoyant density readings.

Some of the earlier bacterial density studies [*Baldwin and Wegener*, 1976; *Poole*, 1977; *Woldringh et al.*, 1981; *Kubitschek et al.*, 1983] have focused on the cell cycle of *Escherichia coli*. The density range of 1.06–1.13 Mg m^{-3} was reported in this literature. This previous research was concerned with possible variations in *E. coli* buoyant densities resulting from different growth stages and growth rates. In the present study, such potential variations in buoyant densities were not investigated. In addition, we did not experiment with bacteria under starving conditions which may be common in subsurface environments. Under starving conditions we acknowledge that buoyant densities, as well as sizes and surface properties of bacteria, may be different.

No previous studies on subsurface bacteria appear to have included measurements of buoyant densities. In the present study of 25 subsurface bacterial strains, buoyant densities ranged from 1.040 to 1.121 Mg m^{-3}. Of these strains, 90% exhibited densities greater than 1.064 Mg m^{-3}. These results span a range similar to that of the previously cited works. The present data demonstrate that bacterial densities are probably commonly significantly greater than that of typical nonsaline groundwaters and suggest that sedimentation is a potentially important mechanism for bacterial transport in groundwater systems.

Stokes' Law Free Sedimentation Velocities and Sedimentation Through Water Columns

Measured bacterial sizes (average lengths and ranges for major and minor axes) of the two strains selected for sedimentation experiments were obtained from microphotographs. This information was used to calculate effective friction factors, assuming prolate ellipsoid shapes. Average major and minor axes for *Arthrobacter* B672 were 1.3 and 0.9 μm, respectively. For OYS3, these values were 5.5 and 2.6 μm, respectively. Calculated free sedimentation velocities for characteristic sizes of these two strains are 5.8 and 40 mm d^{-1}, respectively.

Table 2. Sedimentation Rates

	Arthrobacter B672	OYS3
Buoyant density, Mg m^{-3}	1.117 ± 0.007	1.074 ± 0.009
Major axis length, μm	1.3 (0.8–1.8)	5.5 (3.1–6.2)
Minor axis length, μm	0.9 (0.7–1.0)	2.6 (1.8–3.5)
Calculated free sedimentation rate, mm d^{-1}	5.8 (3.0–8.1)	40 (17–64)
Measured free sedimentation rate, mm d^{-1}	7.1 (5.9–10)	42 (35–47)
Sedimentation rate through Accusand sand, mm d^{-1}	7 (4.5–11)	17 (5.7–22)

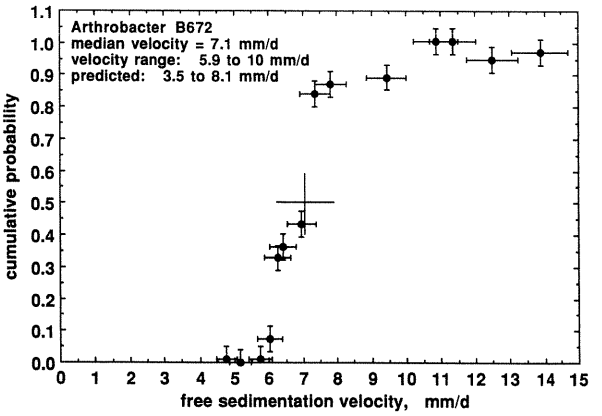

Figure 5. Free sedimentation velocities of *Arthrobacter* B672 in water.

Calculated characteristic sedimentation rates and expected ranges are provided in Table 2.

Results of free sedimentation experiments are shown in Figures 5 and 6. Sedimentation rates were determined by dividing the sedimentation depth (10.0 mm) by the breakthrough times. In these figures the time dependence of normalized 480-nm absorbances of *Arthrobacter* B672 and OYS3 was converted into cumulative probability distributions in a manner analogous to conventional sedimentation particle size analysis [*Gee and Bauder*, 1986]. The slower sedimentation rates of 5.9 to 10 mm d^{-1} were obtained for the *Arthrobacter* B672. The OYS3 strain exhibited higher sedimentation rates ranging from 35 to 47 mm d^{-1}. Comparisons between measured and calculated free sedimentation rates are provided in Figures 5 and 6, as well as in Table 2. Generally good agreement was obtained between calculated and measured characteristic free sedimentation rates for the two bacterial strains. Differences between calculations and measurements may be attributed to uncertainties in sizes and densities and differences between actual shapes and prolate ellipsoids.

Additional influences on the dispersion of the absorbance versus time data can add to uncertainties in calculated sedimentation velocities. These include optical dispersion from the apparatus and Brownian motion. Resolution of the velocity distribution is inherently limited by both of these factors. In the first case the relative uncertainty in velocity is approximately equal to the ratio of the light path slit width (0.5 mm) to the free sedimentation length (10.0 mm). The second source of additional dispersion is due to Brownian diffusion. For a monodispersed suspension of particles with diameter D, the magnitude of diffusional broadening may be estimated by substituting the Stokes-Einstein diffusivity into the error function solution to an initially sharp front [*Crank*, 1975]. The size- and time-dependent result is

$$\Delta z \approx \left(\frac{2kT}{3\pi\eta D^t}\right)^{1/2}, \qquad (12)$$

where k is the Boltzmann constant and T is the Kelvin temperature. For $D = 1$ μm and $t < 2$ days, Δz is smaller than 0.4 mm. Since diffusional broadening is inversely related to particle size, this effect appears to be less than the cuvette slit width (0.5 mm) for experiments on both bacterial strains.

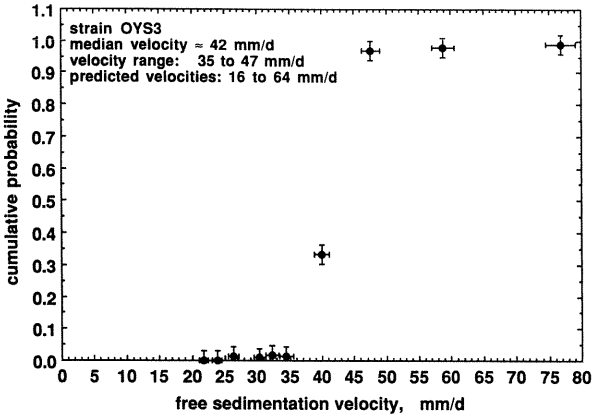

Figure 6. Free sedimentation velocities of OYS3 in water.

Sedimentation Through Saturated Sand Columns

The bacterial strains tested did break through the sand columns under hydrostatic and nonnutrient conditions. All the absorbance data collected (except one of the 20-mm columns, which leaked during the experiment) were corrected for slight decay of optical densities of standard bacterial solutions over the experimental period and then normalized to the absorbance for the case of 100% breakthrough. The breakthrough curves for *Arthrobacter* B672 and strain OYS3 are plotted in Figures 7 and 8, respectively. In both of these figures the upper groups of curves are for bacterial breakthrough from 20-mm-long columns, and the lower groups of curves were from 50-mm-long columns. Average sedimentation velocities through the sand columns were calculated by dividing the average sedimentation distance by the time at which half of the breakthrough occurred. The average sedimentation distance in a column is equal to the length of its sand pack plus 5 mm (the initial elevation of the center of mass of the bacteria relative to the top of the sand pack). Also indicated in each of these figures are time spans associated with free sedimentation range (denoted f.s.r.) for breakthrough in equivalent nontortuous systems. The short-time limit of the f.s.r. for a sand pack of length L is equated with $L/(v_0(\max))$, where $v_0(\max)$ is the measured maximum free sedimentation rate. The long-time limit for a particular f.s.r. is equated with $(L + 10\text{ mm})/(v_0(\min))$, where $v_0(\min)$ is the measured minimum free sedimentation rate. An additional 10 mm is added to the previous result to account for the fact that the longest sedimentation trajectories begin at the tops of the upper reservoir rather than at the tops of sand packs.

The *Arthrobacter* B672 sedimentation through sand is shown as a set of normalized breakthrough curves in Figure 7. The sedimentation rates of the recovered *Arthrobacter* B672 ranged from ≈4 to 11 mm d^{-1}, with a characteristic rate of about 7 mm d^{-1}. Minimum velocities were estimated by dividing the sum of a given column length plus 10 mm by the time needed to obtain 95% relative breakthrough (relative to the plateau in the breakthrough curve). Maximum sedimentation velocities in the sand columns were estimated by dividing a given column length by the time at which 5% relative breakthrough was observed. The approximate median velocities of recovered bacteria are indicated in this figure. Note that both the characteristic rate and range are quite similar to the measured *Arthrobacter* B672 free sedimentation ranges, indicated by the f.s.r. along the time axis. The two f.s.r. in this figure correspond to time ranges needed for free sedimentation bacterial breakthrough in equivalent 20- and 50-mm nontortuous media. The similarities in free sedimentation and porous media sedimentation rates suggest relatively minor grain influences on velocities. The apparently faster upper limit sedimentation velocity in the 50-mm sand column (11 mm d^{-1}) in comparison with the value for free sedimentation (10 mm d^{-1}) is probably within experimental error. A contribution toward increasing the sedimentation velocities in these systems comes from diffusional broadening, which at the 4.5-d initial breakthrough time amounts to a $\Delta z \approx 0.6$ mm. This contribution would provide the leading edge of the free sedimentation case with a rate of 11 mm d^{-1}. Another informative feature of the sedimentation breakthrough curves in Figure 7 is the mass recovery at long times. In the case of the 20-mm-long sand columns, 84 ± 4% of the bacteria were recovered. These results yield deposition coefficients (from (11)) of 0.009 mm^{-1}. It appears that the 55 ± 3% recoveries obtained from the 50-mm columns is incomplete and that these experiments were terminated too early. Using these results a deposition coefficient of 0.012 mm^{-1} is obtained. If the deposition coefficient from the 20-mm columns is used to predict mass recovery for 50-mm columns, a final mass recovery of 64% is predicted.

For strain OYS3, median sedimentation rates through the 20-mm and 50-mm sand columns were 19 ± 1 and 16 ± 2 mm d^{-1}, respectively, as shown in Figure 8. The sedimentation rates for OYS3 through the sand columns are about 40% of its

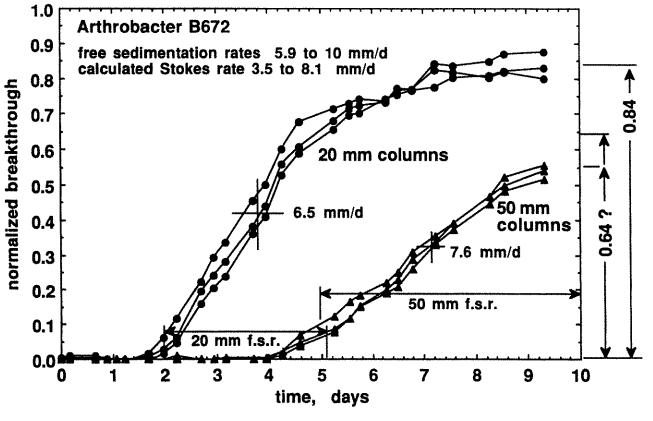

Figure 7. Breakthrough curves for *Arthrobacter* B672 in Unimin sand.

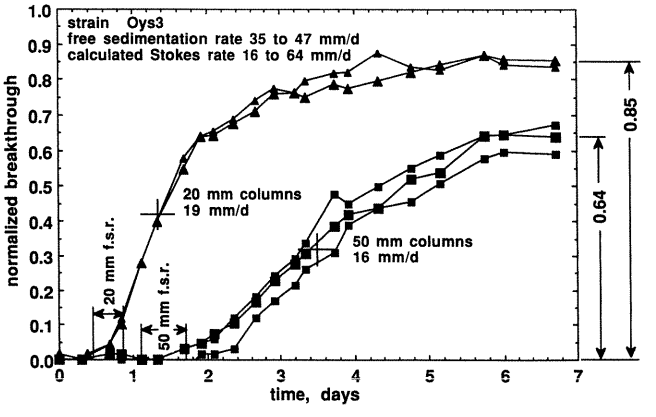

Figure 8. Breakthrough curves for OYS3 in Unimin sand.

free sedimentation rate. Note that the f.s.r. for OYS3 in these columns barely overlap portions of the early breakthroughs in Figure 8. This indicates stronger bacterial-grain interactions with OYS3, compared with *Arthrobacter* B672. For strain OYS3, 85 ± 2% and 64 ± 5% recoveries were obtained in the 20-mm and 50-mm sand columns, respectively. These results yield deposition coefficients of 0.008 and 0.009 mm^{-1}, respectively. An average deposition coefficient of 0.0085 mm^{-1} may be assigned to strain OYS3 sedimenting through Accusand.

The similarities in the sedimentation deposition coefficients for the two bacterial strains are probably coincidental. For other combinations of bacteria, porous media, and solution chemistries, it is expected that wide variations in sedimentation deposition coefficients will be obtained. Our preliminary experiments on bacterial sedimentation in more mineralogically and chemically complex systems indicate that sedimentation deposition coefficients can be much larger than 0.02 mm^{-1} and also exhibit greater variability between replicate columns. Additional studies are needed before the model associated with (11) can be regarded as general to bacterial sedimentation.

Upon termination of experiments involving sedimentation through sands, water samples were extracted from above, within, and below the sand packs for microscopic observations. The intent of these observations was to check for contamination by growth of other bacterial strains and to check for changes in morphology of *Arthrobacter* B672 and OYS3. No contamination by other bacteria was detected. Very few bacteria were observed in waters collected from above the sands. Occasionally, bacteria were detected in samples obtained from within sands. No effort was made to determine depth distributions of retained bacteria. Water samples collected at the bottoms of columns contained abundant quantities of the introduced bacteria; they exhibited no apparent changes in morphology and seemed viable. No tendency for aggregation of bacteria (which would significantly enhance sedimentation effects) was detected in the microscope observations of the postexperiment solutions.

Conclusions

This study demonstrates that sedimentation within pore waters can be an important mechanism for downward transport of bacteria in groundwaters. Although we have investigated sedimentation as a mechanism for bacterial transport, the results have implications on transport of inorganic colloids as well. Sedimentation may be especially important over long periods of time, through many generations of bacteria. In groundwater systems dominated by horizontal flow, sedimentation may contribute to a downward translation of bacteria relative to solute tracers. Unlike diffusion and transverse dispersion, bacterial sedimentation may proceed in direct proportion to time (rather than as a square root of time process), thus having stronger long-term implications. Simple model calculations on well-rounded granular media indicate that in the absence of sorption and frictional effects, bacterial sedimentation through porous media can proceed at rates which are close to (≈90% that of) free sedimentation values. Experimental work supported the general hypothesis and yielded three subsidiary results. First, free sedimentation of groundwater bacteria through static water columns is measurable and in general agreement with Stokes' law calculations based upon direct measurements of buoyant densities and sizes. Measured characteristic free sedimentation rates for *Arthrobacter* B672 and OYS3 of 7 and 42 mm d^{-1}, respectively, indicate that sedimentation velocities are potentially quite significant. Second, sedimentation rates of bacteria through porous media will generally be slower than their free sedimentation rates. In clean, well-rounded quartz sand, sedimentation rates were in good agreement with the simple model. Third, bacterial breakthrough by sedimentation exhibited trends consistent with (although not proving) first-order attenuation with distance. Bacterial sedimentation and attenuation in porous media are expected to be strongly dependent on solution chemistry and surface properties of grains, grain coatings, and bacteria. It is expected that bacterial sorption and adhesion to fixed surfaces in porous media will often be effective in greatly diminishing sedimentation as a transport mechanism. It also needs to be noted that other features of this laboratory-based study may not adequately represent the field environment. For example, laboratory-cultured bacteria may differ from their field counterparts with respect to density, size, and surface properties.

Acknowledgments. We would like to express our appreciation to Anthony V. Palumbo, Anne Happel, Aaron Mills, David Powelson, Thomas Kieft, and Larry Halvorsen for providing us with bacterial strains, sand samples, and helpful discussions. We also thank our colleagues Stefan Finsterle, Anne Happel, and Lea Cox for careful internal reviews of the manuscript and their suggested improvements. The suggestions provided by Ron Harvey and an anonymous reviewer for the final *Water Resources Research* manuscript are much appreciated. Portions of this work were supported through the U.S. Department of Energy contract DE-AC03-76SF-00098 and the Laboratory Directed Research and Development Program of Lawrence Berkeley Laboratory.

References

Baldwin, W. W., and W. S. Wegener, Selection of synchronous bacterial cultures by density sedimentation, *Can. J. Microbiol.*, *22*, 390–393, 1976.

Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas, Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep southeast coastal plain subsurface sediments, *Appl. Environ. Microbiol.*, *55*, 1058–1065, 1989.

Berg, H. C., *Random Walks in Biology*, expanded ed., Princeton Univ. Press, Princeton, N. J., 1993.

Corapcioglu, M. Y., and A. Haridas, Transport and fate of microorganisms in porous media: A theoretical investigation, *J. Hydrol.*, *72*, 149–169, 1984.

Corapcioglu, M. Y., and A. Haridas, Microbial transport in soils and groundwater: A numerical model, *Adv. Water Resour.*, *8*, 188–200, 1985.

Crank, J., *The Mathematics of Diffusion*, 2nd ed., Clarendon, Oxford, 1975.

Fontes, D. E., A. L. Mills, G. M. Hornberger, and J. S. Herman, Physical and chemical factors influencing transport of microorganisms through porous media, *Appl. Environ. Microbiol.*, *57*, 2473–2481, 1991.

Fredrickson, J. K., R. J. Hicks, S. W. Li, and F. J. Brockman, Plasmid incidence in bacteria from deep subsurface sediments, *Appl. Environ. Microbiol.*, *54*, 2916–2923, 1988.

Gannon, J. T., V. B. Manilal, and M. Alexander, Relationship between cell surface properties and transport of bacteria through soil, *Appl. Environ. Microbiol.*, *57*, 190–193, 1991.

Gee, G. W., and J. W. Bauder, Particle-size analysis, in Methods of Soil Analysis, 1, 2nd ed., *Agronomy*, *9*, 383–411, 1986.

Gerba, C. P., C. Wallis, and J. L. Melnick, Fate of wastewater bacteria and viruses in soil, *J. Irrig. Drain. Div. Am. Soc. Civ. Eng.*, *101*(IR3), 157–174, 1975.

Haldeman, D. L., and P. S. Amy, Bacterial heterogeneity in deep subsurface tunnels at Ranier Mesa, Nevada Test Site, *Microb. Ecol.*, *25*, 183–194, 1993.

Harvey, R. W., Parameters involved in modeling movement of bacteria

in groundwater, in *Modeling the Environmental Fate of Microorganisms*, edited by C. J. Hurst, pp. 89–114, Am. Soc. for Microbiol., Washington, D. C., 1991.

Harvey, R. W., and S. P. Garabedian, Use of colloid filtration theory in modeling of bacteria through a contaminated sand aquifer, *Environ. Sci. Technol.*, *25*, 178–185, 1991.

Harvey, R. W., L. H. George, R. L. Smith, and D. R. LeBlanc, Transport of microspheres and indigenous bacteria through a sandy aquifer: Results of natural- and forced-gradient tracer experiments, *Environ. Sci. Technol.*, *23*, 51–56, 1989.

Hornberger, G. M., A. L. Mills, and J. S. Herman, Bacterial transport in porous media: Evaluation of a model using laboratory observations, *Water Resour. Res.*, *28*, 915–938, 1992.

Huysman, F., and W. Verstraete, Effect of cell surface characteristics on the adhesion of bacteria to soil particles, *Biol. Fertil. Soils*, *16*, 21–26, 1993.

Jang, K.-L., P. W. Chang, J. E. Findley, and T. F. Yen, Selection of bacteria with favorable transport properties through porous rock for the application of microbial-enhanced oil recovery, *Appl. Environ. Microbiol.*, *46*, 1066–1072, 1983.

Jassby, A. D., The ecological significance of sinking to planktonic bacteria, *Can. J. Microbiol.*, *21*, 270–274, 1975.

Jenneman, G. E., M. J. McInerney, and R. M. Knapp, Microbial penetration through nutrient-saturated berea sandstone, *Appl. Environ. Microbiol.*, *50*, 383–391, 1985.

Kieft, T. L., P. S. Amy, F. J. Brockman, J. K. Fredrickson, B. N. Bjornstad, and L. L. Rosacker, Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites, *Microb. Ecol.*, *26*, 59–78, 1993.

King, H. E., Jr., E. Herbolzheimer, and R. L. Cook, The diamond-anvil cell as a high pressure viscometer, *J. Appl. Phys.*, *71*, 2071–2080, 1992.

Kubitschek, H. E., W. W. Baldwin, and R. Graetzer, Buoyant density constancy during the cell cycle of *Escherichia coli*, *J. Bacteriol.*, *155*, 1027–1032, 1983.

Lindqvist, R., and G. Bengtsson, Dispersal dynamics of groundwater bacteria, *Microb. Ecol.*, *21*, 49–72, 1991.

Martin, R. E., E. J. Bouwer, and L. M. Hunna, Application of clean-bed filtration theory to bacterial deposition in porous media, *Environ. Sci. Technol.*, *26*, 1053–1058, 1992.

National Research Council, *In Situ Bioremediation: When Does It Work*? Natl. Acad. Press, Washington, D. C., 1993.

Palumbo, A. V., R. S. Burlage, S. P. Scarborough, and J. F. McCarthy, Selection of native strains for bacterial transport studies, paper presented at the 94th General Meeting of the American Society for Microbiology, Las Vegas, Nev., May 23–27, 1994.

Pedersen, K., The deep subterranean biosphere, *Earth Sci. Rev.*, *34*, 243–260, 1993.

Pedersen, K., and S. Ekendahl, Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden, *Microb. Ecol.*, *20*, 37–52, 1990.

Pedros-Alio, C., J. Mas, J. M. Gasol, and R. Guerrero, Sinking speeds of free-living phototrophic bacteria determined with covered and uncovered traps, *J. Plankton Res.*, *11*, 887–905, 1989.

Pertoft, H., and T. C. Laurent, The use of gradients of colloidal silica for the separation of cells and subcellular particles, in *Modern Separation Methods of Macromolecules and Particles*, vol. 2, edited by T. Gerritsen, pp. 71–90, Wiley-Interscience, New York, 1968.

Poole, R. K., Fluctuations in buoyant density during the cell cyle of *Escherichia coli* k12: Significance for the preparation of synchronous cultures by age selection, *J. Gen. Microbiol.*, *98*, 177–186, 1977.

Rajagopalan, R., and R. Q. Chu, Dynamics of adsorption of colloidal particles in packed beds, *J. Colloid Interface Sci.*, *86*, 299–317, 1982.

Rajagopalan, R., and C. Tien, Trajectory analysis of deep-bed filtration with the sphere-in-cell porous media, *AIChE J.*, *22*, 523–533, 1976.

Scholl, M. A., and R. W. Harvey, Laboratory investigations on the role of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer, *Environ. Sci. Technol.*, *26*, 1410–1417, 1992.

Sharma, P. K., M. J. Mcinerney, and R. M. Knapp, In situ growth and activity and modes of penetration of *Escherichia coli* in unconsolidated porous materials, *Appl. Environ. Microbiol.*, *59*, 3686–3694, 1993.

Shonnard, D. R., R. T. Taylor, A. Tompson, and R. B. Knapp, Hydrodynamic effects on microcapillary motility and chemotaxis assays of *Methylosinus trichosporium* OB3b, *Appl. Environ. Microbiol.*, *58*, 2737–2743, 1992.

Stenstrom, T. A., Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles, *Appl. Environ. Microbiol.*, *55*, 142–147, 1989.

Tien, C., and A. C. Payatakes, Advances in deep bed filtration, *AIChE J.*, *25*, 737–759, 1979.

Wan, J., J. L. Wilson, and T. L. Kieft, Influence of the gas-water interface on transport of microorganisms through unsaturated porous media, *Appl. Environ. Microbiol.*, *60*, 509–516, 1994.

Woldringh, C. L., J. S. Binnerts, and A. Mans, Variation in *Escherichia coli* buoyant density measured in percoll gradients, *J. Bacteriol.*, *148*, 58–63, 1981.

Wolff, D. A., The separation of cells and subcellular particles by colloidal-silica density centrifugation, in *Methods in Cell Biology*, vol. 10, edited by D. M. Prescott, pp. 85–104, Academic, San Diego, Calif., 1975.

Yao, K-M., M. T. Habibian, and C. R. O'Melia, Water and waste water filtration: Concepts and applications, *Environ. Sci. Technol.*, *5*, 1105–1112, 1971.

Yates, M. V., and S. R. Yates, Modeling microbial fate in the subsurface environment, *CRC Crit. Rev. Environ. Control*, *17*, 307–344, 1988.

T. K. Tokunaga, C.-F. Tsang, and J. Win, Earth Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, Berkeley, CA 94720. (e-mail: jwan@lbl.gov)

(Received November 25, 1994; revised March 20, 1995; accepted April 17, 1995.)